WATSON

Notes on Orthopteran Gregarinidae with Especial Reference to two Unnamed Species

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NOTES ON ORTHOPTERAN GREGARINIDAE WITH ESPECIAL REFERENCE TO TWO UNNAMED SPECIES

BY

MINNIE ELIZABETH WATSON

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I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

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BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF MASTER OF SCIENCE in Zoölogy

Henry B. Ward
In Charge of Major Work

Henry B. Ward
Head of Department

Recommendation concurred in:

Committee on Final Examination

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INTRODUCTION

The object of this paper is the setting forth of observations and date concerning several species of Gregarinida, parasitic Protozoa of the class Sporozoa, which infest various genera of Arthropoda. Two of the species observed and either a variation of one of the others or another species have not been hitherto described so far as has been ascertained.

HISTORICAL

Gregarines were the first Sporozoa to be recognized but they are still among the least studied forms. The first, but doubtful, record of Gregarine parasitism was made by Redi in 1708 and Ramdohr and Gaede found the parasites in the alimentary canal of insects in 1811 and 1815; but the name Gregarina was to the order which included these parasites by Dufour in 1837, when he described and illustrated six enteric species found in Coleoptera. He regarded them as worms, allied to Trematodes. Dufour found that Gregarines live only in vegetable feeding animals, i.e. those which eat fresh leaves, pollen grains or plant sap, or which feed on decayed wood. He found them in Myriapoda, Annelida and the following Insecta: Coleoptera--Carabidae and Coccinellidae, Orthoptera--Blattidae, Forficulidae, Gryllotalpinae (Mole Crickets), and Gryllidae.

In 1838, Hammerschmidt described several species and subdivided the order into five genera, with, as Leidy says, very little reason.

In 1845, Henle wrote at length on the morphology and movements of a Polycystid form and concludes "I am in doubt whether Gregarines are animals or plants. The navicellae and

even the motion as present in many lower plants supports the latter view."

Kölliker, in 1848, regarded "this singular helminth" as a simple, single organic cell. This statement was doubted by Henle (1845) and Frantzius, who thought the cell complex but vegetable in nature. Kölliker answered the query "Sind die Gregarinen Thiere?" by regarding the "integument of the animal as cell-membrane, the fluid and granular part as cell content and the clear corpuscle within as nucleus with nucleolus." Henle had contended that the corpuscle within the membrane was not a nucleus. The division into two parts (as evinced in Polycystids) Kölliker regarded as of secondary importance, for the septum "consists of the same clear, tough fluid which binds the content of the granules together." Frantzius, in 1848, said the septum is a partition from the general integuments of the body. Kölliker said the membrane contractility and solubility in acetic acid indicate the animal nature, since no contractile cell-membrane soluble in acetic acid is known among plants. Leidy criticised this view by stating that the spores of Achlya, after escaping from the sporangium, indicate a very evident degree of contractile movement in their membrane. Kölliker concludes his argument in 1849 by considering the Gregarine "with its structureless membrane, single content and nucleus in the highest degree like a common cell." He evidently regarded a "common cell" as animal in nature. Leidy

(1853) added to this argument the statement "My observations on the genus Gregarina of Dufour lead me to consider it as occupying a much higher position among helminths than has generally been attached to it, and with Frantzius, Stein and Henle, as not being a simple organic cell."

Leidy thought Gregarines were probably the larval condition of some "more perfect animal","but," he says, "I have not been able to detect any form which could be derivable from them. In the state in which Gregarines are found, they would probably hold a rank between Trematoda and Trichina."

Thus the parasites were considered Protozoa by some writers, and others considered them the embryonic stage of Nematodes, particularly of the genus Filaria. In time this theory fell into disrepute and any resemblances between Gregarines and Nematodes, it became evident, were purely accidental or superficial.

In 1875, Aime Schneider listed the four genera then known and added thirteen new ones. He also described the known species and habitats, contributing twelve species himself. Many well-known forms such as Stylorhyncus found in Coleoptera, Clepsidrina in Blattidae, Porospora in the American lobster, and Actinocephalus in Coleoptera and Myriapoda are included in his list. Schneider also described in detail the morphology of Gregarines and studied their life-history and development. He says "Gregar-

ines are rare in Ascidia, Holothuroidea, Arachnida, and Crustacea; less rare in Turbellaria, Chaetopoda and Cephyra; and rather abundant in Insecta and Myriapoda. Monocystids inhabit the alimentary canal or the coelom and are rare in Arthropoda, Polycystids being limited to the intestinal tract of Arthropoda."

Sporozoa were considered of no importance until about 1875 when the discovery of blood parasites brought these organisms into the foreground. In 1879, a number of isolated unicellular parasites that produce spores with shells were brought together by Leuckart and grouped in the large class Sporozoa. Included with them was the order Gregarinida. It is, however, within the last fifteen years that the entire life-history of Gregarines has become accurately known and "largely in consequence of renewed investigations upon them stimulated by the interesting discoveries made in other orders of Sporozoa."(Minchin, 1903).

CLASSIFICATION

The first attempt at classification of the parasites which Dufour, in 1828, characterized as belonging to a "new and distinct genus of entozoa under the name "Gregarina" was made in 1838 by Hammerschmidt. He described a number of species which, according to Leidy, he subdivided with very little reason into five genera.

Stein (1848) classified Gregarines with relation to the character of the epimerite, as follows:

> Sporadina, not in pairs, head embedded in the intestinal epithelium, short, rounded epimerite. Found by Randohr in 1800 in the Reduvididae (Hemiptera).
>
> Stylorhyncus, sharp backward pointing hooks on the epimerite. Found in the alimentary tract of Agrion (Coleoptera).
>
> Actinocephalus, short epimerite in the shape of a flower. Found in the alimentary tract of Lucanidae (Coleoptera).

This classification was based on an adult morphologic feature. Gabriel (1830) is of the opinion that classification should be based upon the characteristics of the reproductive phase of life history. He thinks that the presence or absence of a septum (which differentiates Polycystids and Monocystids) is an insufficient basis, since one species, Polycystid as an adult, is in its early stages septumless. The apparatus for attachment in

Polycystids is not sufficient for classification, for it is developed in Monocystids also. He has therefore divided Gregarines into two sub-divisions, (1) Acystoplasta, wherein encystation occurs during the reproductive process, and (2) Cystoplasta, where it does not, i.e. wherein spore formation is complete without encystation and without alteration of any sort in the shape of the body.

Bütschli (1882) classified Sporozoa in Bronn's Klassen und Ordnung and his work has been made the basis for subsequent classifications. He divided the class Sporozoa into these sub-classes: Gregarinida, Myxosporidia and Sarcosporidia. The first is again divided thus:

Order 1. Monocystidea. Gregarines without differentiation of the body into two or more parts by a transverse septum.

Order 2. Polycystidea Schneid. 1872. Gregarines differentiated into deuto- and proto- merite with epimerite in young forms.

Genus Duforia Schneid. 1875.

Bothriopsis Schneid. 1875.

Porospora Schneid. 1875.

Stenocephalus Schneid. 1875.

Hyalospora Schneid. 1875.

Euspora Schneid. 1875.

Clepsidrina (Hammerschm.) Schneid. 1875.

Pileocephalus Schneid. 1875.

Echinocephalus Schneid. 1875.

Stylorhyncus (Stein 1848) emend. Schneid. 1875.

Geneiorhyncus Schneid. 1875.

Actinocephalus (Stein 1848) Schneider.

Pyxinia Hammerschm. 1838, Schneider.

Léger's classification of Sporozoa, somewhat altered by Wasielewski (1896) is as follows:

Class Sporozoa
Order 1. Gregarinae
2. Haemosporidia
3. Coccidida
4. Acystosporidida
5. Myxosporidida
Sarcosporidia
Amoebosporidia
Serosporidia

the last three forming an appendix, their classification being uncertain. Gregarinae are further subdivided on the basis of the presence or absence of a spore-covering thus:

Sub-order 1. Gymnosporeae, without spore-covering
2. Angiosporeae, with spore-covering

The former possesses small round spores, and contains one unfamiliar family. The latter includes the Polycystides and Monocystidea and is next subdivided into groups of spores with like and unlike poles. The further divisions are still more detailed. Angiosporeae embrace nine families and includes all the common species.

Labbé (1899) classifies Sporozoa somewhat differently, and because his classification is a recognized standard today,

is given below in some detail.

Legion 1. Cystosporidia
2. Myxosporidia
Sarcosporidia
Amebosporidia
Serumsporidia

the last three being Sporozoa of uncertain classification.

Cystosporidia (rarely ameboid, constant in form, mononucleate, often epithelio-intracellular, with encystment stage preceeding sporulation) are further subdivided as follows:

Order 1; Gregarinida (Sporulation non-intracellular, habitat intestinal- or body- cavity)
Sub-order A. Cephalina (Possessing epimerite at some stage in life history, protomerite and deutomerite, associative, chiefly intestinal parasites of Arthropods)
Tribe 1. Gymnosporea (Without spore-covering)
Family i. Aggregata (Approximate closely Gregarinida but have alternation of generations associated with change of hosts)
ii. Porosporidae (One unfamiliar species, parasitic in the lobster)
Tribe 2. Angiosporea (Possess spore-covering)
Family i. Didymophyidae (Associative, no septum between primite and satellite)
ii. Gregarinidae (Associative, trophozoite with simple epimerite, oval spores)
iii. Dactylophoridae (Asymmetric, epimerite)
iv. Actinocephalidae (Solitary, epimerite, symmetric, pointed, no spore ducts)
v. Acanthosporidae (Solitary, epimerite symmetric, stalked or rosetted, no spore-ducts)
vi. Menosporidae (Long stalked epimerite, crescentic spores)
vii. Stylorhynchidae (Long stalked epimerite, spores round and in chains)
viii. Dolicystidae (Oval spores)
Sub-order B. Acephalina (Includes the Monocystidea of the seminal vesicles of Lumbricus, Lankesteria of Tunicates, and species in Holothuroidea and Echiuroidea)

Order 2. Coccidida
3. Haemosporidiida
4. Gymnosporidiida

The family Gregarinidae is characterized thus: Adults either solitary or in association, epimerite symmetric, simple. Cysts with or without spore-ducts, spores round or oval. There are eight genera, as follows, with twenty-six species listed in the group up to 1899:

Gregarina Dufour, associative, epimerite small, rounded, host-genera Forficula, Gryllus, Blatta, Tenebrio (larva)? Ephemera, Lepisma, etc.

Ganocystis Aime Schneider, associative, temporary epimerite. Parasitic in insects.

Hirmocystis Leger, epimerite papillar, associative, two or three individuals together, spherical cyst, without spore-ducts, oval spores, enteric. Host genera Limnobatidae (Hemiptera), Tipulidae (Diptera).

Hyalospora Aime Schneider, solitary, protomerite sub-globular, deutomerite cylindrical, elliptoid spores. Rare.

Euspora Aime Schneider, solitary, protomerite often missing, prismatic spores. Rare.

Chenidospora Aime Schneider, solitary, protomerite sub-globular, deutomerite cylindrical, elliptoid spores. Rare.

Stenophora Labbe, protomerite small, deutomerite larger, parasites with a uniform, unsymmetric body, possessing no epimerite, mostly coelom parasites, several species found in gut of Diplopods.

Doflein (1911) follows the general order as above for the families of the tribe (sub-legion) Angiosporea of Leger except that he raises Labbé's genus Stenophora to a family. He, however, classifies Sporozoa somewhat differently, making two

sub-classes Telosporidia and Neosporidia, the former with reproduction at the close of the life-history, i.e. with the use of all the protoplasm of the two individuals in the production of spores within the cyst, the latter with reproduction at intervals during the adult stage. Telosporidia include the orders Coccidiomorpha (sub-orders Coccidia and Haemosporidia) and Gregarinida. The latter order is subdivided into Eugregarinida and Schizogregarinida.

Eugregarinida (Léger) are typically without schizogony, reproduction being limited to sporulation. Two individuals unite in the formation of a spore, copulation being isogamous or anisogamous. Spores contain eight sporozoites. They include the Monocystidea which possess an epimerite in no part of their life-history and Polycystidea which possess epimerite at some stage during their life-history. The latter, in turn, are divided into Gymnosporea, which lack spore covering, and Angiosporea, which possess same. Doflein's further classification follows closely that of Labbé. Calkins (1909) bases his classification of Sporozoa upon that of Labbé, modified slightly by that of Minchin.

From characteristics of the adults, trophozoites and early stages of the cyst, the writer would place the species described in this paper in the family Gregarinidae, genus Gregarina (Dufour). They show external characteristics similar to this

genus. The sporonts are rarely found solitary, but ordinarily exist in associations of never more than two. The epimerite is rarely present in sporonts or trophozoites. When present, it does not in all three cases conform to the typical conical papilla of the genus Gregarina Dufour, but may be a simple, serrated, truncated cone set into the center of the anterior end of the protomerite of the primite. One instance was observed inwhich the epimerite was the typical rounded papilla. (Fig. 12).

The forms could possibly be classified in only one other genus of the family Gregarinidae, Hespora, (Lebé, 1899), but an epimerite has never been described for this genus, and the sporonts are found both solitary and in association, while in the forms studied associations of two are the rule.

Therefore, since (1) the described new forms are almost without exception associative when in their normal condition (they easily break apart when hardened in alcohol), and since (2) an epimerite is found, although not conforming strictly to the type-form, the writer feels justified in placing the new species under the genus Gregarina Dufour.

MATERIAL

The material for the new species of Gregarines described in this paper was collected in the vicinity of Urbana, Illinois, during the months of September, October, November, and December, Nineteen twelve and May, Nineteen thirteen.

Two fields were regularly visited, one just north of Crystal Lake, a timothy meadow, the other a clover field south of the Forestry. Other places were sporadically visited, including woodlands, vacant lots, roadsides and open fields.

The host-species examined for parasites included:

Annelida: Lumbricus terrestris

Myriapoda: Julus canadensis
Lithobius ? sp.

Coleoptera: Carabidae (unidentified species)
Galuita janus

Diptera: various larvae

Lepidoptera: various larvae

Orthoptera: Blatella germanica
Blatta orientalis
Gryllus pennsylvanicus
Gryllus domesticus
Encoptolophus sordidus
Dissosteria carolina
Xiphidium ? sp.
Melanoplus acrididum
Melanoplus differentialis
Schistocerca americanum
Arphia sulphurea
Hesperotettix praetensis
Melanoplus femur-rubrum

METHODS

The protozoan parasites in Orthoptera were studied both alive and after fixation. Living forms were studied by snipping off mouth and anal regions of the host and drawing out the alimentary tract upon a glass slide. The tube was then slit lengthwise. The parasites were found to lie in all parts of the tract, generally, however, lying in the region between the pyloric caeca and the Malpighian tubules, either free-living or mingled with the undigested food or attached by the anterior end to the epithelium. Cysts were found free and imbedded in faeces in various parts of the alimentary tract.

The living forms were studied both in the normal digestive juices diluted slightly with water and in normal salt solution. The latter caused the otherwise sluggish movements to be accelerated, the parasites moving rapidly away from the common center by two forms of motion to be described later. In both media the animals ultimately disintegrated, the walls bursting by osmotic pressure, with consequent extrusion of the protoplasmic content.

Two fixing agents were used with equally good results, cold corrosive-acetic and Schaudinn's mixture of two parts saturated aqueous corrosive sublimate, one part absolute alcohol

and a trace of acetic acid.

Ehrlich's acid haematoxylin was used as a stain both for whole mounts and for sections. Picro-carmine and borax-carmine were used for whole mounts and Heidenhain's iron haematoxylin for sections, the best results being procured with the first and last named, nuclear details being thus clearly delineated.

Whole mounts were prepared in the following manner: Individual specimens were isolated with a capillary pipette with as little as possible of the culture medium and placed in a depression slide. Corrosive-acetic was added, and after a few minutes the specimens were transferred to another slide and 75% alcohol added. A slide was smeared with egg-albumen and the gregarines transferred to this. After the alcohol had coagulated the albumen, the slide was transferred to a jar of 75% alcohol and subsequently treated as a section mount.

Whole mounts were also made after carrying the material through the alcohols in the usual manner. The region parasitised was cut from the host's alimentary tract and the whole treated with corrosive-acetic for five minutes, washed with 70% iodine-alcohol, then transferred to 70% alcohol for preservation until used. The free parasites are heavy and sink to the bottom of the vessel, thus being readily isolated from the less dense tissues. They were placed on slides only after being stained and cleared in xylol.

Section mounts were made in paraffin, cut at seven microns in thickness.

EXPLANATION OF TERMS

The terms used in the following description which are mostly peculiar to Gregarines are as follows (Minchin, 1903):

Monocystids, forms without an epimerite and septum separating the body.
Polycystids, forms with an epimerite, and which are divided by a septum into protomerite and deutomerite.
Primite, the first individual of an association.
Satellite, the second individual of an association.
Epimerite, the organ of fixation to host-tissues, present on the anterior end of the protomerite of the primite.
Protomerite, the first, small, chamber of an extracellular Gre Gregarine, separated by a septum from the larger
Deutomerite, the distal portion which contains the nucleus.
Epicyte (Ectosarc, Cuticle), an ectoplasmic secretion of some thickness, often produced into hooks or spines on the epimerite.
Sarcocyte, the superficial layer of ectoplasm just within which is found a deeper layer, the
Myocyte, consisting of contractile fibrillae, for use in motion.
Endocyte, the protoplasmic body-content within the ectoplasm.
Trophozoite, the individual parasite during the stage of rapid growth and absorption of food from the host.
Cephalont, the young trophozoite while attached to the host-walls by the epimerite.
Sporont (or Gametocyte), the free adult trophozoite when mature, for reproduction.
Gamete, one of the small nucleated masses into which the Gametocyte breaks up, constituting the first step in the reproductive process.
Zygote, or definitive sporoblast, the union of the two gametes for the production of spores.
Spore (or Pseudonavicella, from the form-relation to the diatom navicella), the metamorphosed zygote.
Cyst, the common envelope surrounding the two associated gametocytes.
Epicyst, the outer cyst-covering.
Endocyst, the thinner, inner layer of the cyst.
Sporocyst, the tough integument surrounding each spore.
Sporozoite, one of the eight nucleated divisions of a ripe spore.
Syzigy, a chain formed by a primite and one or more satellites attached end to end, the anterior end of one adhering to the posterior end of the other.

RECORD OF OBSERVATIONS

A. TABLE INDICATING OBSERVED INSTANCES OF GREGARINE PARASITISM

Host-species	Date of examination	Estimated number of gregarines	Organ inhabited	Condition of parasites	Remarks
Julus canadensis	Oct. 7	10	Alim.tract	Sporonts	
	Oct. 8	0			
Lithobius ? sp.	May 4	100	"	Sporonts and trophozoites	
Carabidae (6)	Oct. 11	0			
Carabidae (2)	May 5	0			
Diptera (larvae) (25)	May 5	0			
Galuita janus (27)	Oct. 11	0			
" " (2)	May 5	0			
Lepidoptera larvae (3)	Oct. 7	0			
Lumbricus terrestris (5)	Nov. 4	800	Seminal vesicles	"	Monocystis (species unidentified)
Blatella germanica (6)	Dec. 20	0			Nematode infection
Blatta orientalis (15)	"	0			"
Gryllus pennsylvanicus	Sept. 20	40	Stomach	"	
" (5)	Oct. 5	10-30 each	"	"	
Gryllus p.	Oct. 14	15	"	"	
Gryllus domesticus	Oct. 7	0			
" "	"	10	"	"	Also infected with Gordius
" "	"	10	"	"	
" "	"	0			

Encoptolophus sordidus	Nov. 16	5	"	Sporonts, trophozoites and cyst	Two species of Gregarines	
"	Nov. 20	100	"	"	"	
"	Nov. 22	20	"	"		
" (2)	Nov. 24	50	"	"		
"	"	0				
Dissosteria carolina (2)	Oct. 14	20 each	"	Sporonts, trophozoites		
Xiphidium ?sp.	Oct. 10	10	"	"		
"	Oct. 10	10	"	"		
"	Oct. 16	10	"	"		
Melanoplus acrididum (2)	Oct. 16	10 each	"	"		
Melanoplus differentialis	Oct. 7	500	Whole alim. tract	"		
"	Oct. 10	10	Stomach	"		
"	"	100	Stomach and intestine	Sporonts, trophozoites and cysts	Host dead, twenty-two cysts in dense excreta mass in intestine	
"	Oct. 17	25	"			
" (10)	Oct. 18	25-300 each	"	Sporonts and trophozoites		
"	Oct. 18	50	"	" and cysts		
"	Oct. 28	25	Stomach	"		
"	Oct. 28	0			Two long Gordiaceae	
"	Nov. 2	1000	Whole alim. tract	"	Two sp. of Gregarines, larger sp. predominating. Fifteen cysts of equal size One cyst	
"	Nov. 8	6	Stomach	"		
"	Nov. 12	200	"	No cysts		

Schistocerca americana		May 6	1	Stomach	Sporont	
Arphia sulphurea		May 7	0			
" "		"	0			
Hesperotettix pnaetensis		May 7	40	Intestine	Sporonts and tropho-zoites	
"	(3)	"	0			
"		"	100	Rectum	"	Embedded in mass of ex-creta
"		"	2	Stomach	Sporonts	
"		"	100	"	"	
"	(3)	"	0			
Melanoplus femur-rubrum	(6)	Oct. 10	50 each	"	Sporonts and tropho-zoites	
"		"	500	Whole alim. tract	"	
"	(24)	Oct. 11	10-500 each	"	"	All infected
"	(14)	Oct. 14	10-200 each	"	"	"
"	(13)	Oct. 16	20-100 each	"	"	
"		"	300	"	"	Gordius infection also
"		Oct. 17	1	Stomach	Sporont	Fly larvae infection also
"		Oct. 17	500	Whole alim. tract	Sporonts and tropho-zoites	
"		Oct. 17	250	"	" and cysts	Twenty-five cysts, est.
"	(13)	"	50-200	"	No cysts	All infected
"		Oct. 21	500	"	Sporonts and tropho-zoites	
"		"	300	"	"	Cysts
"		"	300	"	"	
"	(4)	"	200 each	"	" and cysts	
"		"	0			
"	(6)	"	10-50	"	"	
"		Oct. 28	0			
"		Oct. 31	25	"	"	

Melanoplus femur-rubrum		Oct. 31	85	Whole alim. tract	Sporonts and trophozoites Cysts	Two species of Gregarines thirty five of smaller, fifty of larger sp. Two large cysts, two half-size of former. One Gordius
"		Nov. 2	25	Stomach	Sporonts and trophozoites	Two days after first hard freeze; No cysts. Parasites practically motionless
"		Nov. 4	5	"	" and cysts	Host dead. Parasites nearly transparent. Three cysts
"		Nov. 5	10	"	Sporonts and trophozoites	Host dead two days when examined. No cysts. Less opaque than usual, nucleus plainer Gordius infection
"	(2)	Nov. 7	15-25 each	"	"	
"	(3)	Nov. 7	40-50 each	"	" and cysts	
"		"	50	"	"	
"		"	1000	Whole alim. tract	"	Whole alim. tract densely crowded. Many cysts. Several shapes and sizes. After heavy frost and several days of cold rain
"		Nov. 9	150	"	"	Five cysts. Two sp. of

					parasites
Melanoplus femur-rubrum	Nov. 12	200	Whole alim. tract	Sporonts and tropho-zoites	
"					
"	Nov. 14	500	"	"	
"	"	50	"	" and cysts	Five cysts. Host dead. Parasites but slightly opaque
"	Nov. 15	25	Stomach	Sporonts and tropho-zoites	
"	Nov. 16	50	"	"	
"	Nov. 16	10	"	" and cysts	
"	"	10	"	"	Two cysts
"	Nov. 18	50	"	No cysts	
"	Nov. 20	300	"	Sporonts, tropho-zoites and cysts	
"	Nov. 26	100	"	"and cysts	Two sp. of Gregarines
"	"	0	"		One cyst

Notes on table:

The fall dates refer to 1912; the spring to 1913.

Numbers in parenthesis after host-species indicate that several similar individuals were examined the same day and grouped together.

By stomach, as organ inhabited, is included the region of the alimentary tract between the gastric caeca and Malpighian tubules.

Conclusions from the foregoing table:

(1). Percentage of infection is high. Tabulated, it appears as follows:

Host	Number examined	Number parasitized	Percentage of infection
Julus canadensis	3	1	33 1/3%
Lithobius ? sp.	1	1	100%
Carabidae	8	0	0%
Diptera (larvae)	25	0	0%
Galuita janus	29	0	0%
Lepidoptera larvae	3	0	0%
Lumbricus terrestris	5	5	100%
Blatella germanica	6	0	0%
Blatta orientalis	15	0	0%
Gryllus pennsylvanicus	6	6	100%
Gryllus domesticus	4	2	50%
Encoptolophus sordidus	6	5	83 1/3%
Dissosteria carolina	2	2	100%
Xiphidium ? sp.	3	3	100%
Melanoplus acrididum	2	2	100%
Melanoplus differentialis	20	19	95%
Schistocerca americana	1	1	100%
Arphia sulphurea	2	0	0%
Herperotettix praetensis	10	8	80%
Melanoplus femur-rubrum	115	113	98.3%

(2). Percentage of infection increases as the season advances and is much lower in the spring than in the autumn months.

(3). Number of parasites in an individual host is much less in the spring than in the fall.

(4). Individual parasites are pearly white in the spring and grayish, tan or brown in the fall.

(5). At least two new species of Gregarinidae appear in the Acrididae hosts, as follows:

Encoptolophus sordidus
Dissosteria carolina
Xiphidium
Melanoplus acrididum
Melanoplus differentialis
Schistocerca americana
Arphia sulphurea

Hesperotettix praetensis
Melanoplus femur-rubrum

(6). Different species of parasites are not confined to particular hosts, but all the Acrididae examined were susceptible to the several species found.

(7). When more than one species of Gregarinidae is found in the same host, one form predominates over the other in numbers.

(8). Sporonts infrequently occur embedded in masses of excreta in the rectum, but more frequently free in the alimentary canal.

(9). Cysts are often embedded in masses of excreta within the rectum, indicating their extrusion and development in the open.

(10). Death of the host does not cause the parasites to at once encyst. The parasites die after the host has been dead two or three days.

(11). Sudden decrease in temperature above the freezing point does not cause the parasites to become encysted.

(12). After the first heavy frost, no more encysted forms were found than before.

(13). Advance of the season seems to have little to do with frequency of cysts.

(14). Heavy infection apparently exerts no deleterious effect on the host, two or more Gordiaceae often occurring in the coelom of a host whose alimentary tract is densely crowded with Gregarinidae.

(15). The digestive tract often contains much of the brown digestive fluid, and the parasites are invariably more numerous under such condition than when little or none of this juice is present.

RECORD OF OBSERVATIONS

B. TWO NEW SPECIES OF GREGARINIDAE IN ACRIDIDAE

The hosts which harbored these gregarines include those mentioned under 'Material', a species of Gregarinidae not being confined to any particular host.

Gregarina obesa (See Figs. 1 and 2)

Average length: .272 mm.

Maximum length: .36 mm.

Degree of infection: Generally very abundant. Numbers of individuals vary from (estimate) ten to five hundred or more per host.

Region of infection: When present in small numbers, the stomach, i.e. alimentary tract between gastric caeca and Malpighian tubules; when present in large numbers, the whole alimentary tract.

Condition of individuals: Sporonts and trophozoites. Associations of two the rule. Single individuals, including trophozoites, rare.

Table of dimensions (Individuals taken from many hosts, at different parts of the season):

Length of primite	Length of satellite	Length protomerite of primite	Length protomerite of satellite	Width protomerite of primite	Width protomerite of satellite	Width deutomerite of primite*	Width deutomerite of satellite*
.24 mm.	.27 mm.	.061 mm.	.03 mm.	.13 mm.	.14 mm.	.175 mm.	158 mm.
.29	.295	.062	.032	.10	.10	.20	.17
.27	.305	.08	.04	.089	.081	.115	.115
.325	.31	.08	.035	.115	.095	.115	.135
.21	.21	.055	.04	.07	.08	.09	.11
.225	.22	.06	.03	.082	.075	.09	.11
.26	.29	.062	.03	.162	.126	.26	.207
.30	.36	.065	.04	.115	.12	.219	.21
Averages:							
.262	.282	.066	.035	.110	.102	.162	..157

*Greatest width

Epimerite basal portion expanded, terminating in a serrated truncated cone. Rarely present in young forms, never in adults. (Fig. 5).

Protomerite of primite hemispherical, bluntly pointed at distal end. Width at base nearly twice the length. Protomerite of satellite much flattened at top, three times as long as wide as long, with indentations in sarcocyte for attachment of primite. Endocyte of protomerite more dense than that of deutomerite. (Fig. 13)

Deutomerite of primite globose; that of satellite longer and narrower.

Nucleus indicated in cleared specimens, five-sixth the size of the protomerite of primite, spherical, position variable, with no regularity of position in specimens of stated sizes. Contains from one to four or more large, granular, deeply-staining, vacuolated bodies.

Epicyte a very thin, flexible, brittle wall, with fine longitudinal markings (serrations). (Fig. 14).

Sarcocyte a thicker layer, clear, apparently striated, surrounding the whole body; average thickness .008 mm.; thickest at tip of primite protomerite (.01 mm.), at edges of septum between protomerite and deutomerite, and at posterior end of deutomerite (.006 mm.).

Endocyte granular, often vacuolated or reticulated in deutomerite. Dense, nucleus not visible in larger specimens. In vivo, brown in transmitted light in the fall-collected individuals; in the spring pearly white or tan. Smaller specimens less dense, tan in color in the fall, nucleus visible. Protomerite more dense than deutomerite.

Gregarina obtusa (See Figs. 3 and 4)

Average length: .42 mm.

Maximum length: .59 mm.

Region of infection: Same as for Gregarina obesa

Condition of individuals: Associations of twos the rule. Single individuals not uncommon, probably due to insufficient care in technique. Primites and satellites readily distinguishable when separated. (Fig. 3).

Table of dimensions (Individuals taken from many hosts, at various parts of the season):

Length of primite	Length of satellite	Length protomerite of primite	Length protomerite of satellite	Width protomerite of primite	Width protomerite of satellite	Width deutomerite of primite*	Width deutomerite of satellite*
.42 mm.	.43 mm.	.107 mm.	.09 mm.	.095 mm.	.09 mm.	.17 mm.	.14 mm.
.40	--	.12	--	.11	--	.158	--
.43	.42	.115	.105	.08	.07	.145	.155
.365	.44	.10	.035	.085	.11	.14	.13
.31	--	.08	--	.08	--	.13	--
.49	--	.10	--	.14	--	.18	--
.375	--	.085	--	.09	--	.13	--
.34	--	.08	--	.09	--	.13	--
.41	.39	.14	.05	.10	.08	.15	.15
.35	--	.07	--	.10	--	.15	--
.28	--	.09	--	.07	--	.10	--
.36	--	.10	--	.09	--	.14	--
.39	--	.10	--	.10	--	.15	--
.29	--	.10	--	.09	--	.12	--
.50	--	.18	--	.14	--	.11	--
.25	--	.07	--	.08	--	.11	--
.56	--	.17	--	.14	--	.15	--
.50	--	.18	--	.14	--	.15	--
.37	--	.10	--	.09	--	.13	--
.51	--	.09	--	.09	--	.15	--
.36	--	.09	--	.11	--	.14	--
.51	.49	.16	.11	.12	.11	.14	.12
.59	.52	.16	.11	.14	.13	.15	.17
.49	.44	.10	.05	.11	.09	.16	.15
.42	.35	.11	.09	.11	.10	.16	.13

Agerages:

.411	.435	.111	.08	.104	.097	.145	.146

* Greatest width

Epimerite present in one-fourth the specimens examined, serrated. (Figs. 6, 7, 8).

Protomerite of primite dome-shaped, higher than wide, slightly wider at base than at end. Sporonts possess cup-shaped indentation at apex. (Fig. 11). Protomerite of satellite slightly compressed at anterior end; sarcocyte containing indentation into which fits posterior end of deutomerite of primite. (Fig. 13).

Deutomerite two and one half times as long as wide, widest part anterior half; posterior half terminating in a bluntly pointed cone. Deutomerite of satellite averaging longer than that of primite by .05 mm.

Nucleus spherical or nearly so, averaging .04 mm. in diameter. More densely granular than cytoplasm but not visible in vivo through cell-walls. Contains two or more vacuolated, deeply-staining bodies. (Figs. 17 and 18).

Epicyte thin, brittle, flexible, marked with fine longitudinal ridges. (Figs. 14, 15, 16, 17).

Sarcocyte a clear, striated (?) layer surrounding the body. Slightly thicker at anterior end of protomerite of primite and at edges of septum separating protomerite and deutomerite. (Figs. 15 and 16).

Endocyte granular, dense, dark brown in transmitted light, small and starved specimens being less dense, with nucleus visible. (Figs. 15 and 16).

Gregarina sp. var. cylindriatus

(See Figs. 19 and 20)

The forms classified under this heading were found in fewer numbers than the two species above, and not confined to a particular species of host. A few were measured, and the results given below:

Length of primite	Length of satellite	Length protomerite of primite	Length protomerite of satellite	Width protomerite of primite	Width protomerite of satellite	Width deutomerite of primite	Width deutomerite of satellite
.27 mm.	.305 mm.	.08 mm.	.04 mm.	.089 mm.	.08 mm.	.11 mm.	.115 mm.
.325	.31	.08	.095	.11	.095	.13	.135
.21	.21	.05	.04	.07	.08	.09	.11
.32	.36						
.286	.25						
.255	.31						
.35	.28						
Averages:							
.288	.289	.07	.038	.089	.085	.116	.12

Locomotion and morphology approximate that given for the other forms.

The writer is unable, at the present time, to state whether this form is a variant of Gregarina obesa or of Gregarina obtusa, or a distinct species. It will be seen from the table immediately following that it differs but slightly in length from Gregarina obesa, either in protomerite or deutomerite, the chief difference being in the width of the deutomerite. Thus, the general body proportions more closely approximate those of Gregarina obtusa.

Table of Comparison of the Average Measurements of the Three Forms

	Length primite	Length satellite	Length protomerite of primite	Length protomerite of satellite	Length deutomerite of primite	Length deutomerite of satellite	Width protomerite of primite	Width protomerite of satellite
Gregarina obesa	.262 mm.	.282 mm.	.066 mm.	.035 mm.	.196 mm.	.247 mm.	.110 mm.	.10? mm.
Gregarina obtusa	.410	.435	.111	.08	.299	.35	.104	.0?
Gregarina sp. var. cylindriatus	.288	.289	.07	.038	.218	.231	.089	.0??

	Width deutomerite of primite	Width deutomerite of satellite
Gregarina obesa	.162 mm.	.157 mm.
Gregarina obtusa	.146	.146
Gregarina sp. var. cylindriatus	.116	.12

MORPHOLOGY

The first to describe in detail morphologic features of the genus Gregarinidae was Henle, who, in 1845, said Gregarines are cylindrical, divided into head, neck and body, that they have a homogeneous content with a nucleus and one or more nucleoli. He said the nucleus has a varying content, thus substantiating what he says Von Siebold found, viz."instead of a single nuclear body (nucleolus), several small scattered bodies or several bodies united together to form a twisted, worm-like thread."

Stein, in 1848, detailed the morphology of Polycystids. The outer covering, the cuticle, he described as a glassy, transparent, smooth, elastic covering, structureless and homogeneous. There may be an unarmed projection from the head end for grasping. Stein says the cuticle absorbs liquids, this being the only method of ingesting food; hence Gregarines swell in water until they break, and canals and dark masses of protoplasm then show inside as though specialized organs. They were also regarded as such by Dufour in 1828 and even by Van Beneden in 1872.

Van Beneden (1872) differentiated four body layers, a cuticla, a thin muscular-fibrillar-layer running transversely, a slimy, clear, longitudinally striated and non-muscular

cortical parenchyma, and a granular central parenchyma. He thus locates the muscular layer in the outer ectoderm instead of embedded in the outer layer of the endoderm.

Schneider (1875) distinguishes epicyte (cuticla), sarcocyte, myocyte, endocyte, nucleus, septa between protomerites and deutomerites, and appendicular organs (hooks, etc.) at the anterior end. The outer covering, the cuticla, he describes as a glassy, transparent, smooth, elastic covering, conforming to Stein's description. The sarcocyte may be absent and the fibrillar element therein not always developed. Contractility does not reside in the myocyte, and is independent of the presence of the fibrillar layers.

The cuticle (epicyte) (Fig. 15, a) is a membranous secretion of the ectoplasm. It is found to be a delicately ribbed structure with meridional striations running from pole to pole. (Fig. 14,b). Schewiakoff (1895) notes that there is always a transparent layer between the sarcocyte and the cuticle proper, in motile forms, and from this region a series of narrow longitudinal slits opens upon the bottom of the longitudinal furrows of the cuticle, thus furnishing a means of exit for the jelly-like substance. He found that slight pressure caused drops of exude to form and smear the whole exterior of the body with a viscous jelly. Wasielewski (1896) says the ectoplasm is composed of three layers, epicyte, sarcocyte and

myocyte, with a layer between the epicyte and sarcocyte for the 'Gallertfaden' exudation.

Inside the cuticle is found the living ectoplasm, the sarcocyte, (Fig. 15,b), a clear striated hyaline layer of varying thickness, and imbedded in the inner parts thereof are contractile fibrils, constituting the myocyte. The myonemes, or fibrils of the myocyte, run around the body and are seen only in longitudinal sections. This myocyte was first observed by Van Beneden, (1873) and then by Schneider (1875). Van Beneden describes the myonemes as small dense granules lying in rows; Schewiakoff (1895) says they anastamose along the sides of the body so that the covering appears to be a network of circular fibrillae. The sarcocyte, in any particular cross-section may vary in thickness (Fig. 15), it may vary with different specimens (Compare Fig. 15, x 645 and Fig. 16 x 800) or it may vary in different regions of the body, being thicker at the anterior end of the protomerite and generally slightly thicker at the posterior end of the deutomerite than in other parts. (Figs. 1, 3, 11, 13, 19, etc.) The septum separating proto- from deuto- merite is a thin layer of sarcocyte (indicated in cleared mounts, Figs. 2 and 4).

The endoplasm (Fig. 15, c) is a viscous fluid, non-vacuolated and densely granular especially in adult forms, when the opacity is marked, the color ranging from white to dark brown. The number of protoplasmic granules increases as the

animal grows, causing the opacity of the adult. Sometimes the consistency is homogeneous throughout; just as often it is very heterogeneous, clear sap-filled spaces alternating with the dense, irregularly shaped, brownish masses of protoplasm.

The consistency of the adult sporont is varied. Prenzel (1892) differentiates in the whole body content as many as fourteen substances, as follows:

1. Protpelastin in the cuticle, epimerite, septum and nuclear wall, insoluble in nitric or acetic acid, ethyl alcohol or chloroform, soluble in alkalis.
2. Alveolin, in the protoplasmic mesh-work. Insoluble in acetic, sulphuric or nitric acids, potassium hydroxide or saliva, fixed by mercuric chloride, chloroform, incative toward iodine.
3. Paralveolin, accompanying (2) but soluble in saliva, acids and alkalis.
4. Neutral fat in drops, especially found in protomerite.
5. Albuminoids, some fixed by mercuric chloride, others by acids.
6. Protocollagen, swelling in acetic acid, shrivelling in water.
7. Paraglycogen in the granules, iodine reaction red-violet with aid of sulphuric or nitric acid. Changed by warm sulphuric into sugar.
8. Pyxinin, the corresponding substance in Pyxinia, changed by acetic or nitric acid into an amorphous substance without iodine reaction.
9. Anti-enzym, a hypothetical substance which hinders digestion.
10. Morubin, the substance of the nuclear-morulite, soluble in nitric but not in acetic acid or enzymes.
11. Paramorulin, the network in the nucleus, fixed by acetic and nitric acids. Digestible.
12. Nuclein in the nucleoli.
14. Cell-sap.

Other authors find fewer constituents. Wasielewski (1896) mentions these: (1) Paraglycogen, chiefly round or oval granules staining with iodine and sulphuric acid, (2) "Warnino-

phile' granules which stain red with picro-carmine , discovered by Schneider; (3) Paxinia granules, characteristic of that genus and therein substituted for 1 above; (4) fat globules; (5) Protein crystals; (6) Unknown compounds varying in different genera of Gregarinida.

Minchin (1903) enumerates these compounds: (1) Large paraglycogen spherules allied to starch and glycogen, staining in iodine, dissolved in alkalis; (2) Carminophilous granules, containing an albuminoid stained by picr-carmine; (3) fat globules; (4) protein crystals, and 'other more or less enigmatic enclosures.'

The endocyte is often seen to be more dense in the protomerite than in the deutomerite, particularly in adult forms. This, however, is not a constant factor.

The position of the nucleus is varied. It lies within the endocyte, generally somewhere near the center of the body, but may be found near either end. During violent motion of the animals, the nucleus changes its position, following the direction of the protoplasmic flow. In very young trophozoites, the nucleus is large compared to size of the body (Fig. 9) but assumes smaller proportions in adults, being approximately the same size in all the specimens measured. A list of such measurements, taken from adults, follows, the specimens being whole mounts.

0.030 mm.
.0299
.034
.04

.039
.038
.04
.036
.044
.039
.046, etc., an average of thirty five measurements being 0.038 mm.

The nucleus is surrounded by a wall, and lies in a clear space in the endoderm, being invisible in live sporonts. It often shows in the trophozoites. One or more karyosomes, spherical vacuolated bodies, isolated, band-like or beaded are present. (Figs. 15, 17, 18.)

MOVEMENTS

The movements of Gregarines were early observed. Henle (1845) described the movement as consisting of a drawing together of the whole body and then outstretching, with consequent displacement of the nucleus. Stein (1848) spoke of most Gregarines as able to execute but weak movements, but some "perform worm-like rapid and contorted movements, when the nucleus is thrust quickly from end to end."

Leidy (1853) observed in Gregarina juli-marginati (Leidy) and in Gregarina Blatta-orientalis (Leidy) that contractility exists only in the deutomerite. "Movement consists of a slow bending or constriction or involution of the deutomerite, or contraction of the protomerite without involution, or general contraction removing any involution, with projection of the cephalic cell if it had been previously retracted." Leidy discovered a tunic "which had entirely escaped the notice of all previous observers", "marked by a most beautiful set of exceedingly regular, parallel, longitudinal lines, present only in the deutomerite." The only clue to the origin of movement, he states, is as follows: "The contractile movements--- appear to be of a muscular character, to such a degree that I was led to the detection of the muscular tunic in seeking for their source." Thus Leidy thinks vermiform contractility and the forward gliding movement as both

caused by the 'muscular tunic.'

Van Beneden (1872), in writing on morphologic features of Gregarines, says the cortical parenchyma possesses longitudinal striations due to ridges and channels of the inner layer, but which are neither muscular nor permanent. He says there is a transverse, thin, true muscular-fibrillar-layer, just without the former. Lankester (1866) criticises Van Beneden's statement concerning the muscular layer: "It is often absent, and when present does not function as a contractile layer." It will be noted that Van Beneden's location for the longitudinal striations in the inner cortex is incorrect, and also his statement that this layer is not a permanent structure.

Schneider (1875) says that contractility does not reside in the myocyte and is independent of the presence of fibrillar layers. Hertwig, in his Manual of Zoology (1912) states this explanation of motility: "There is a double striping of the body, a longitudinal recognizable by furrows on the surface and hence cuticular, and a transverse marking in the ectosarc, produced by circular or spiral muscle fibrillae. These muscles explain the peristaltic motion and the occasional bending of the body, but not the peculiar gliding motions by which locomotion is usually effected. It may be that the gregarines secrete stiff gelatinous threads from the posterior end, and the elongation of these forces the body forward."

Doflein (1911) thinks that Diatomaceae, Desmidiaceae,

Gregarinidae, and Coccidiae are able to glide about without bodily constrictions, but they all secrete much slime which remains in the form of a stalk clinging to the posterior end of an association. He describes the cuticle as a resistant epithelial cover possessing longitudinal ridges with furrows from which the jelly-like exudation takes place. This exudation emanates from the second ectoplasmic layer, the 'Gallertschicht', which lies just beneath the cuticle in all parts of the body, and flows backward in the furrows to the posterior end. This theory which Doflein describes was advanced by Schewiakoff (1895), who found in the study of Clepsidrina that movement is effected by the deposition of a jelly-like substance exuded from the body at the posterior end of the deutomerite, in the form of a stalk. Thisstalk readily congeals, adheres to the surface upon which the gregarines lie, and the continuous exudation and growth of the stalk forces the parasites forward. Sokolow (1912) developed this theory in detail. He divides gregarines into six groups, according to the kind and degree of movement, thus:

1. Motionless, without myoneme, without jelly-layer.
2. Vermiform movement, myoneme layer, no jelly-layer.
3. Energetic vermiform movement, weakly developed myoneme, no jelly-layer.
4. Energetic forward movement, weakly developed myoneme, jelly-layer.
5. Slow, gliding forward movement, weakly developed myoneme, jelly-layer.
6. Forward gliding and vermiform movement, well-developed myoneme, jelly-layer.

He concludes that those forms without myoneme are motionless; those with a myoneme layer are able to exhibit vermiform move-

ments but not forward movements; those with a jelly-like layer but weakly developed myoneme are able to move forward but not to exhibit well the contraxtile movement, and that the forward movement is correlated with the presence of the jelly-like layer.

Sokolow demonstrated the presence of the stalk by the use of carmine grains, which adhere in clumps behind the gregarine while it is moving forward. When one clump becomes too large to be carried, it is dropped and another begins to collect. The exude is excreted in the form of a long, slender, resistant thread, and these threads, lying interwoven and in disorder, form a hollow cylindrical stalk. Sokolow concludes that the forward movement is not performed primarily through contraction of the myoneme layer, as Crawley indicates (1905) but that such contraction may play a secondary role. He does not think, as does Schewiakoff (1895) that the forward movement is the cause of growth of this jelly-like substance into a hollow cylinder, but that the exudation is thrust out rather energetically backward, and there is a consequent reaction of the animal forward.

The writer has made the following observations regarding movement in the species studied: Two movements are exhibited, (1) a diatom-like gliding forward, slow and steady, and seen for short distances only, (2) a rapid, convulsive bending one individual of an association on the other, or one part of an individual

on another, a torsion of part on part, suddenly begun and as suddenly stopped. After such movement, the individual remains quiescent.

The first movement is probably closely related to the exudation of the jelly-like substance . Carmine grains were observed to adhere sparingly to all parts of the body, but to adhere in masses at the posterior end of the satellite. When one mass is too large to be carried, it is dropped and another is accumulated. By shutting off most of the light and using a magnification of approximately 500 diameters, fine clear longitudinal threads can be observed among the carmine grains. The exterior layer of the body is marked with fine, longitudinal ridges in both protomerite and deutomerite. In cross-section, they might easily be mistaken for short cilia. (See Figs 8, 14, 15, 16, and 17). Doflein (1911) says the troughs of these ridges throughout the body contain minute pores, through which the exudation passes, and from which it flows to the posterior parts in the troughs. Since carmine grains do adhere to all parts of the body, somesticky material must be present throughout. A train of grains half as long as the individual may be carried. Since this gliding-forward movement is intermittent, and since the masses of carmine are at times dropped, the writer is inclined to think that when the animal stops, it is because it has exuded all of the jelly-like substance it has manufactured, and must remain quiescent until more is accumulated. Gregarines have not been observed to glide backward, and no

account of such movement as has been found by the writer. According to the above theory, such movement would be an impossibility.

The rapid, contorted motion seems to be independent of any exudation at the posterior end of the satellite, since both satellite and primite have the powers of contractility. Both may contract at the same time, or independently. Thus some other explanation must exist to account for such movement. Such explanation cannot be stated with certainty, for the writer is not assured as yet of the existence of myonemes. The larger granules of protoplasm in the endosarc cannot be safely differentiated from myonemes as described by Doflein. He states that they are best developed at the junctions of protomerite and deutomerite, but such structures seem to be no more in abundance there than in other parts of the outer layer of the endosarc. The writer is of the opinion, however, that there must be some morphologic basis for this form of movement, and further investigation will be made toward finding such structures by differential staining and by various chemical reactions.

Gregarines are often seen with a shred of tissue attached to the posterior end of the satellite. This indicates one of two things: that the animal has attached itself to the epithelial cell by this end and has subsequently torn a shred loose in freeing itself, or that it has been picked up in the intestinal debris. The exudation serves another purpose than that of for-

ward movement, it serves as a means of attachment for primite and satellite (or satellites). In some species, several satellites are attached end to end. In the ones studies, only one satellite has been observed, with one exception. In this instance three small trophozoites were found attached by their protomerites to the posterior end of a single large sporont.

REPRODUCTION AND LIFE HISTORY

Whether or not the associations of two adult sporonts is the first step in reproduction of Polycystids is still a doubted question. Some investigators think the association is merely mechanical, for adults are seen in strings of several, in twos or alone. Others see in the union a deeper meaning.

Henle, in 1845, observed the union of two individuals. "Die Verbindung scheint durch aufnehme einer Hervorragung des einen in eine Vertiefung des andern Individuums zu geschehen die Trennung erfolgt auf leise Beruhrung und dann sind die Vorderenden beider Individuum von einander nicht zu unterscheiden."

Leidy observes that "in most Gregarines two individuals commonly of the same size conjoin in the same line, the cephalic extremity of one attached to the caudal end of the other."

Von Siebold, in 1839, and Stein, in 1845, stated that Gregarines reproduce from the union of two individuals within a cyst. Stein was the first to assert that the conjugation process is applicable to animals. He says that the cyst is formed from the shortening and rounding up of the two associated individuals. His statements are based on researches on Tenebrio molitor, the larva being the meal worm.

Schneider appended Stein's work by asserting that he has found conjugation to be true for certain species in animals

but that in general cysts are formed from one animal alone, and not from the conjunction of two. Schneider thought encystment took place in Polycystids and Monocystids from one sporont and that the one individual divided during encystment, for he saw the two hemispheres in a young cyst.

It was Bütschli (1882) who first indicated the complete encystment process in the manner understood today. He showed that encystment takes place after the union of two individuals, the individuals thickening up and changing from a lighter to a dense brown just previous to cyst-formation. They no longer move in straight lines, but in large circles, later rotating upon themselves as axes. Circular motion indicates cysts in the early stages of formation and rotation later stages. Change from normal associations into the completely formed cysts may take place in half an hour. A thin, fragile, transparent layer forms around the outside. In half an hour this becomes thick and dense, dark in color and many layered, the layers forming concentric circles. The cyst,up to this time globular, now becomes ovoid (in the species Bütschli describes) and the line of demarkation of the two constituent individuals begins to disappear. Gametes begin to form after forty eight hours, and before the septum between protomerite and deutomerite has been absorbed. The gametes areready for union with those from the other side of the septum before this septum completely disappears. The gametes

he says, are formed by a budding process from the outer layers of the individuals which make up the cyst, but not at the surfaces which are flattened against each other, for this septum later bursts, allowing intermingling of the anisogamous gametes. This stage is reached forty-eight hours after the beginning of cyst-formation. The zygote formed by the union of the two gametes becomes transparent. Butschli did not observe further zygotic changes but goes into detail regarding his theory of the origin of spore-ducts. Stein (1848). he says, first observed the method of scattering of the spores. Later it was observed by Schneider and his views substantiated by Butschli himself. Spore-ducts appear forty-eight hours after complete encystment. The ducts appear to be continuous with the inner cyst-wall. KOH (35%) destroys the non-protoplasmic network granules or renders them transparent, and the protoplasmic network is left, and in this network the spore-ducts become more easily visible. They break through the outer cyst-covering previous to ripening of the spores. The ripe spores are thrust out probably by slight pressure exerted from without inward by the thickening of the outer wall, or from contractile power residing in this outer layer.

Minchin (1903) states that a curious feature of Gregarines is their tendency to form associations during the trophic period. In a form found in the body cavity of the cricket, young trophozoites become associated in couples almost immediately after leaving the host cell, and no solitary individuals are to be

found above a certain size since all the old maids die off. Lankester details the breaking up of the nuclei of the two gametocytes (adult sporontsX, each first splitting mitotically into two then into four parts, and so on, to form the total number of gametes. The gametes of the two individuals are unlike, as Doflein (1911) illustrates for Stylorhynchus and Monocystis (pp. 716) and which Leger calls Macro- and Micro- gametes, or female and male gametes, the latter possessing a flagellated motile apparatus. The female is the smallerand without much reserve material, the male being the larger and stored with much reserve material. "The sporonts may be considered to be potentially male and female, each sporont occupying half of the cyst, so that a male and a female chamber can be distinguished." The two gametes fuse to form a zygote nucleus, which further separates amitotically into two, then four, and finally eight smaller nuclei; each becomes surrounded by a small portion of the protoplasmic mass, and by a thin but resistant wall. The eight sporozoites are now completed. Further changes have not been observed in a single host. The ripe spores (of Monocystis) apparently require transference to a new host for completion of their life-history. The spores getting into a new host, the stomach of a new earthworm, are caused to burst open and scatter thes porozoites by action of the digestive juices, the sporozoites being capable of boring their way through the tissues into the sperm sacs, there developing through the trophozoite stage into adult sporozoites.

The life-history of Gregarines has been worked out most thoroughly in Monocystis, and the steps are seen to include: Sporont- Cyst- Gamete- Zygote- Spore- Sporozoite- Young trophozoite- Free trophozoite- Sporont.

The stages in development are three: (1) a trophozoite stage when nourishment is taken from the host; (2) an encystment stage; (3) a free stage, when the spores are disseminated in the open.

It is probable that infection is accidental. The disseminated spores may be taken up with the food. It seems likely that Acrididae re-infect themselves. They live in restricted areas, probably not venturing far from the habitat field except during long migrations. Eating infected grass and clover, they become many times re-infected throughout the summer. In the late fall, all stages of trophozoite and development were met with in the same host.

The steps in the reproductive process which the writer has observed are as follows:

1. Thickening and increasing opacity of the associated individuals.
2. Bending of the two individuals on each other.
3. Circular motion, when this bending had progressed so that the protomerite of the primite was nearly in contact with the posterior end of the deutomerite of the satellite.
3. Whirling motion of newly formed cyst (in another specimen than the above).

4. Presence of the dark brown, thin cyst covering, the identity of each individual still being retained, i.e. the walls being intact, septa between protomerite and deutomerite still present, both nuclei normal in size and apparent content.

6. Presence of a thick, gelatinous, [illegible]-layered epicyst, which increases as the cyst shrivels. Closer union of the walls separating the individuals.

7. Disappearance of the nuclei.

8. Complete fusion of the two individuals in a cyst, neither septa nor nuclei being visible, cyst apparently homogeneous and dark brown.

9. After the cyst had been removed from the alimentary tract and placed in water medium two days, a crack was noted along one side, with exudation of much of the cyst content, but not in spore condition, rather as protoplasmic granules. This condition was undoubtedly a premature rupture superinduced perhaps by pressure of the cover glass and by the fact that the mounting medium used was water.

The writer is inclined to think that the two individuals constituting an association (in the Polycystids studied) represent physiologically different constitutions, although ripe gametes have not been observed. When mounted in water medium, osmotic pressure causes the walls to burst, but the primite walls break some time before those of the satellite. This is without exception, true in cricket gregarines as well as those in Acrididae, and would indicate a difference in the epicyte thicknesses. It is hoped that chemical analysis of the two will reveal cytologic differences. In a gregarine from a cricket motility was exhibited in primite and satellite in different degrees, the primite becoming contorted while the satellite remained nearly

constant in shape. No case was observed in which the satellite bent as readily nor to as great an extent as the primite.

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EXPLANATION OF FIGURES

Figures are made from camera-lucida drawings and sections are cut at 7 micra

1. Sporont association of primite and satellite, Gregarina obesa, from living forms. x 280.

2. Sporont association of primite and satellite, G. obesa, drawn from whole mount, and showing nucleus visible only after clearing. x 280.

3. Sporont association, G. obtusa, from living forms. x 280.

4. Sporont association, G. obtusa. Whole mount. x 280.

5. Sporont primite, G. obesa, with slightly abnormal epimerite. Whole mount. x 645.

6. Sporont association, G. obtusa, showing serrations on epimerite and association at beginning of rotating movement. From living specimens. x 86.

7. Primite showing epimerite attached to epithelial tissues. From living specimen. Satellite broken off. x 86.

8. Oblique section through primite, G. obtusa, showing serrated epimerite, the sarcocyte with longitudinal ridges on both protomerite and deutomerite and variations in form and distribution of protoplasmic granules. x 800.

9. Young trophozoite, whole mount, showing a) serrations on epimerite, b) large size of protomerite relative to deutomerite in young forms, c) proportionally large nucleus. Compare b and c with same structures in an adult specimen, e.g. Fig. 2 Same magnification as Fig. 2.

10. Young trophozoite, in vivo, showing proportionally large protomerite. x 280.

11. Portion of protomerite of primite to indicate infolding of apex in sporonts after epimerite has fallen off. Also showing relative thickness of parts of sarcocyte in protomerite. x 645.

12. Sporont with papillar epimerite still attached. x 280.

13. Protomerite of satellite dismembered from primite, showing interlocking device for attachment of posterior end of deutomerite of primite. x 645.

14. Oblique sections through deutomerite, showing a) hair-like edges of longitudinall serrations, b) crests and troughs of these serrations (this is almost a longitudinal section, with ridges running from pole to pole), c) infolding at posterior end of deutomerite. x 800.

15. Cross-section through deutomerite, showing a) serrated cuticle, b) sarcocyte of uneven thickness, c) endocyte, d) nucleus with unevenly distributed chromatin. x 645.

16. Segment of cross-section, higher magnification than Fig. 15, showing differentiation in consistency of protoplasmic granules. Sarcocyte a thinner layer than in last. x 800.

17. Cross-section showing vacuolated character of nucleus. x 1000.

18. Nuclei from promiscuous sporonts indicating various stages in chromatin distribution, small isolated bead-like particles, larger isolated particles crescentic and rounded, a chain of bead-like granules, and a thick ribbon-like mass of chromatin. See Figs. 2, 19 and 22 for other chromatic arrangements.

19, 20, 21. Stained associations of G. sp. var. cylindriatus to show individual differences in size, and to show that primite of an association may be larger than the satellite, or vice cersa. In Fig. 21, the section is not cut through nucleus. Sections. x 280.

22. Outline drawing of sporont embedded in loose epithelial tissue. x 280.

23. Cyst in early stage of formation, cyst-wall not being shown. x 280.

24. Cyst in early stage, sporonts not yet in copulation ?. x 280.

25. Stained cyst, similar to Fig. 24, but cleared, and drawn from different viewpoint, showing nuclei and cyst-wall. x 280.

26. Mounted and cleared cyst showing walls not yet fused.
x 280.

27. Cyst with wall of separation beginning to disintegrate.
Alcoholic specimen. x 280.

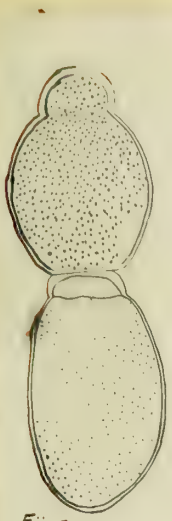

Fig. 1

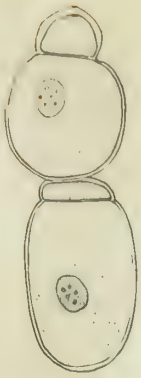

Fig. 2

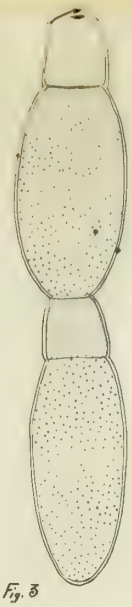

Fig. 3

Fig. 4

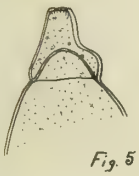

Fig. 5

Fig. 6

Fig. 9

Fig. 7

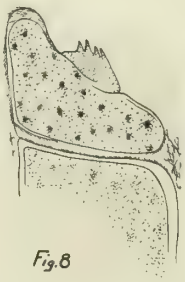

Fig. 8

Fig. 14

Fig. 10

Fig. 11

Fig. 12

Fig. 13

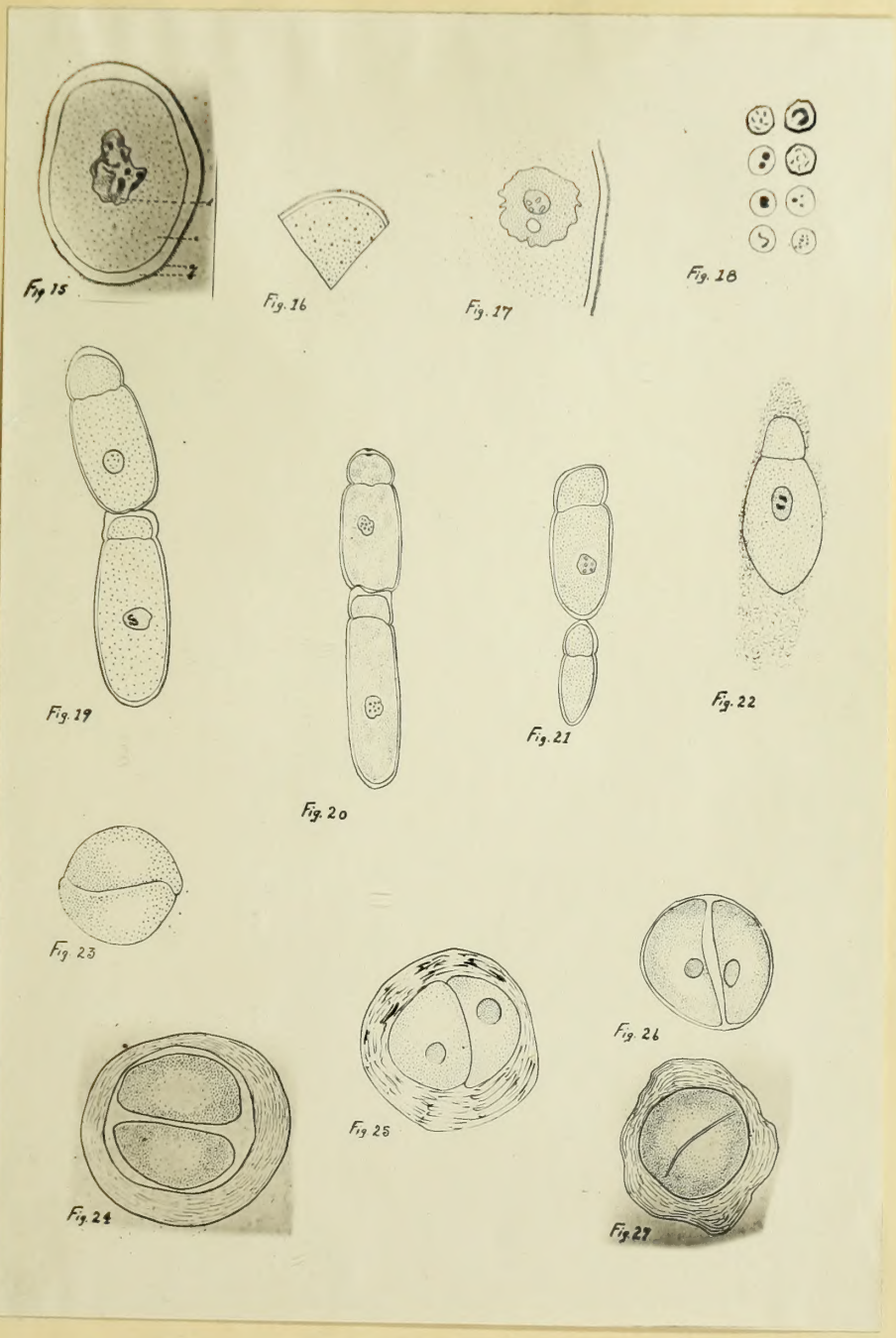
Fig. 15
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